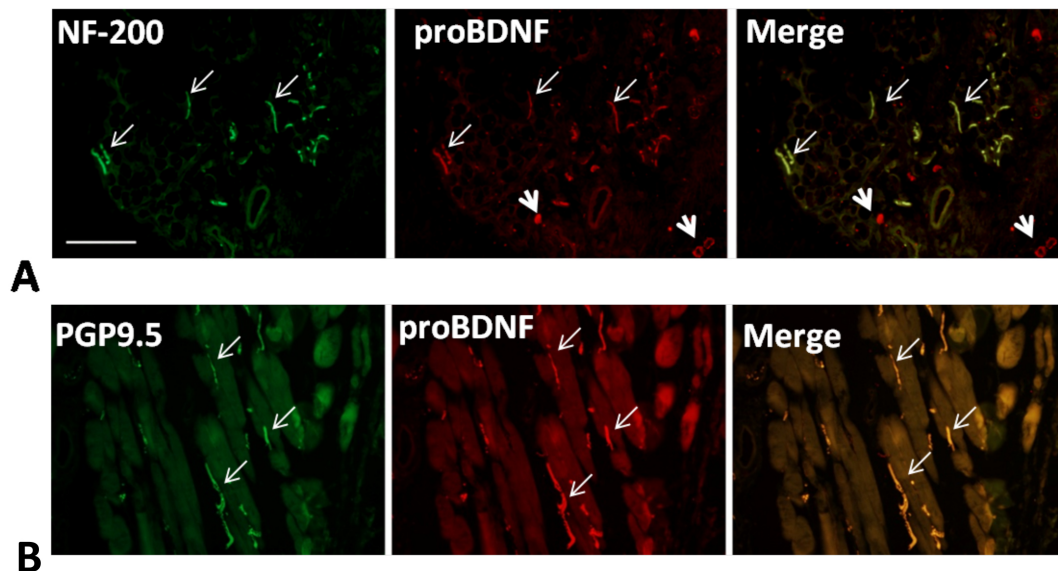

Peripheral Brain Derived Neurotrophic Factor PrecursorRegulates Pain as an Inflammatory Mediator

Running title: peripheral proBDNF regulates pain

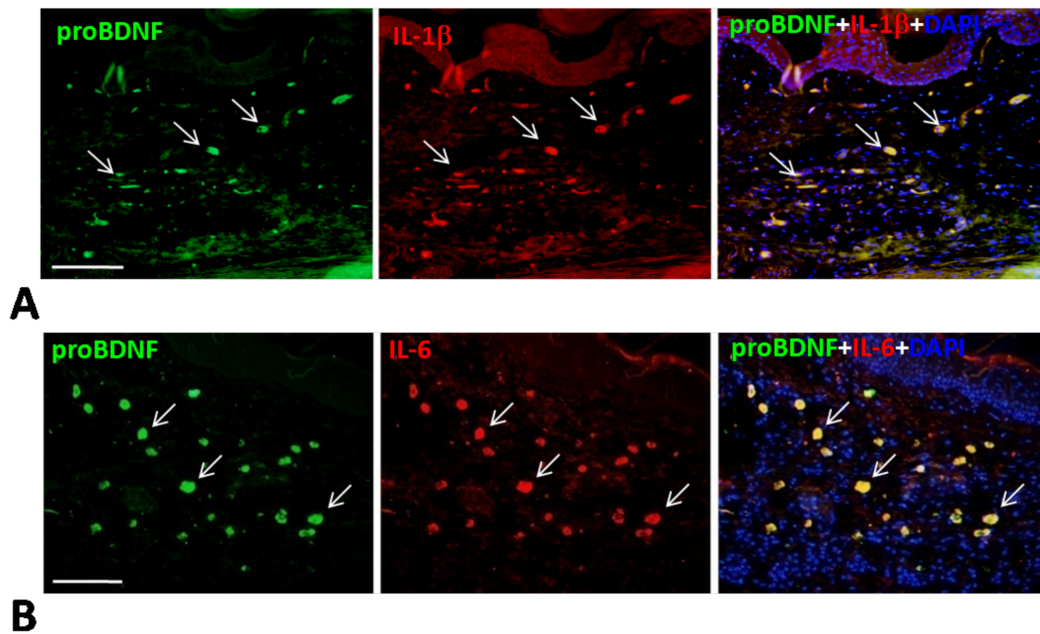
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Supplementary Information

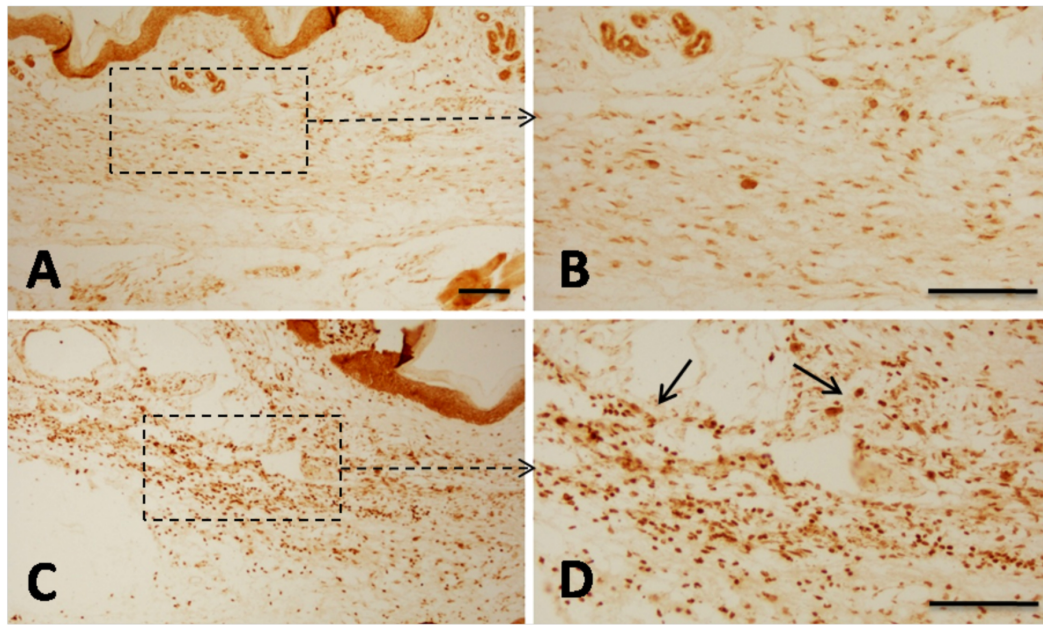


Supplementary Figure S1. Double labeling of proBDNF with nerve fiber markers in the footpad skin after formalin intra-plantar injection. Male Kunming-mice were conducted with unilateral intra-plantar injection with 10ul 5% formalin. At 3 h post-injection, mice were euthanized by over-dose chloral hydrate and processed for double-labeling immunofluorescence. (A) Co-localization of Neurofilament-200 (Green) with proBDNF (Red) after formalin hindpaw injection. Note the abundant co-localization of proBDNF with NF-200 (thin arrows). Some of

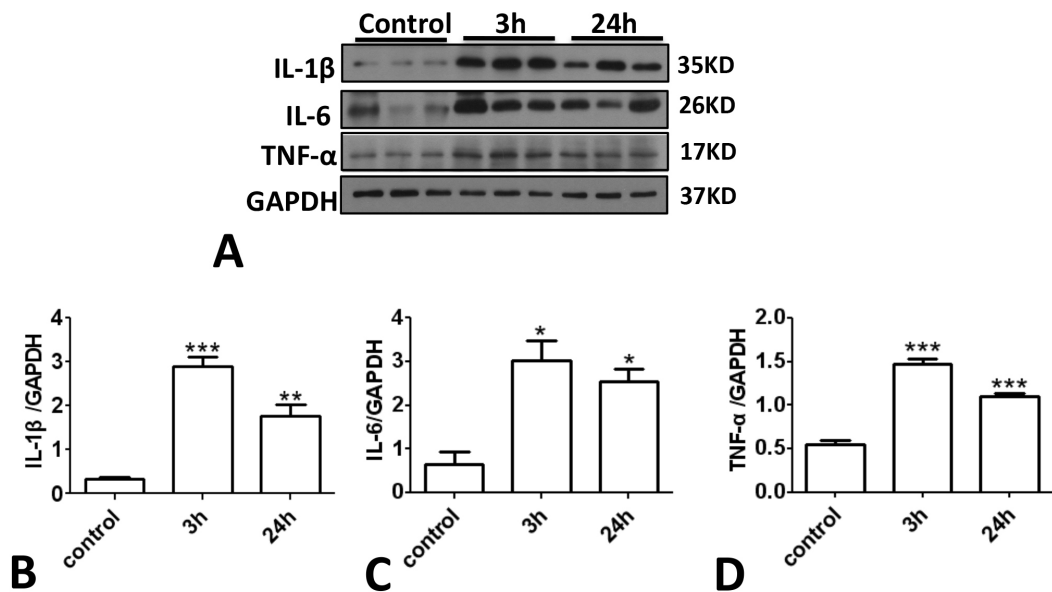
proBDNF positive staining was not expressed in the NF-200 positive staining (thick arrows). **(B)** Double labeling of PGP9.5 (Green) and proBDNF (Red) showing that proBDNF is co-localized with PGP9.5. Scale bar, 50 μ m, 3 replicates, n=3 per group.



Supplementary Figure S2. Co-localization of proBDNF with IL-1 β (A) and IL-6 (B) positive staining cells in response to formalin hindpaw injection. Male Kunming-mice were subjected to 5% formalin intra-plantar injection and euthanized for immunofluorescence at 3h post-injection. Note that co-localization of IL-1 β (A) and IL-6 (B) positive staining with proBDNF positive staining (Arrows). Scale bar, 50 μ m, 3 replicates, n=3 per group.



Supplementary Figure S3. Representative immunohistochemistry of p75NTR in the local tissue in the normal control or at 3 h after formalin intra-plantar injection. In the normal control foot skin, p75NTR immunoreactivity appeared to be localized in the nerve-fiber like structures in the subcutaneous layers (**A** and **B**). After formalin intra-plantar injection, p75NTR immunoreactivity was dramatically increased and mainly expressed in the inflammatory cells (Arrows, **C** and **D**). Scale bar, 50 μ m, 3 replicates, n=3 per group.



Supplementary Figure S4. Western blot of IL-1 β , IL-6 and TNF- α at 3 h and 24 h after proBDNF protein (0.25ug) intra-plantar injection into mice. (A) Representative immunoblot of IL-1 β , IL-6 and TNF- α after proBDNF protein injection. **(B-D)** Statistical analysis of IL-1 β **(B)**, IL-6 **(C)** and TNF- α **(D)** expression (*p<0.05, **p<0.01, ***p<0.001 versus control, one-way ANOVA followed by Dunnett's Multiple Comparison post hoc test, n=3 per group).